# Experimental Lathyrism in Mice Fed Diets Containing Sweet Peas or β-aminopropionitrile.* (23422)

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When β-aminopropionitrile (BAPN) or the seeds of *Lathyrus odoratus* (sweet pea) are fed to rats, an experimental lathyrism is produced which is characterized by skeletal and aortic changes(1-9). There are no reports in the literature concerning similar findings in mice. In fact, it is generally supposed that mice are unaffected by the toxic factor of sweet peas because Lewis and Schulert(10) reported that white mice showed no evidence of toxicity when fed diets containing 50% sweet peas for 17 weeks.

In a preliminary experiment, we fed a diet containing 50% sweet peas to 3 weanling mice. These mice grew normally and showed no obvious skeletal changes. After 4 weeks of feeding, however, 2 animals died suddenly from ruptured aortas. We have, therefore, studied the effect of feeding diets containing sweet peas or BAPN to albino mice to determine the difference in response, if any, between mice and rats to such diets.

*Methods.* Weanling, Swiss albino, male mice, from the mouse colony of the Department of Oncology, were used. The pea diets were made up as previously described(11). The BAPN-containing diets were prepared by drying a solution of synthetic BAPN hydrochloride on a weighed amount of milled Rockland rat stock diet. The BAPN used in these diets was synthesized by the method of Buc (12), converted to the hydrochloride, and crystallized from 95% alcohol. All diets were supplied *ad libitum*. Animals were weighed weekly and autopsied at death or at termination of experiment. For microscopic studies, tissues were fixed in 10% buffered formalin or alcoholic formalin (10% in 95% alcohol). They were dehydrated, cleared and embedded in paraffin. Six to 10 μ sections were stained with hematoxylin and eosin, or a modified Hornowski's combined elastic and Van Gieson method for connective tissues. Selected sections were stained with Lillie's modification of the McManus P.A.S. method.

*Results.* As indicated in Table I, when diets containing 50% sweet peas or 0.2% BAPN • HCl were fed, the mice grew normally. Higher levels of BAPN caused a depression in growth rate in proportion to the level of BAPN fed. Although many deaths occurred suddenly and without warning, on higher levels of BAPN death was sometimes preceded by weight loss.

After 3 weeks, mice receiving 50% sweet peas or 0.2% BAPN • HCl showed none of the symptoms of advanced experimental lathyrism observed in *all* rats consuming such diets for similar periods(3,4). Only after 4 to 7 weeks of feeding did a few of these mice develop palpable spinal curvatures. At autopsy, most animals in these groups had small but well-defined exostoses on the medial aspects of their femurs in contrast to rats which had grossly misshapen femurs in much shorter time intervals(3,4) (Fig. 1). Three of the 44 mice on these diets developed paraphymosis (a common occurrence in rats receiving such diets for similar periods). Twelve of the 44 mice had gross aneurysms of the aorta, 7 of which ruptured causing death of the animals. Deaths from aortic rupture occurred at varying times between the 14th and 68th day after beginning the experimental feedings.

Differences in composition of the sweet pea diets used by us and by Lewis and Schulert (10) would not seem to explain the complete resistance of their mice to the toxic factor. While increased levels of casein have been found to be partially protective against skeletal(3,13,14) as well as aortic lesions(15,16) in experiments with rats, in the present experiments a level of casein which was higher

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TABLE I. Incidence of Gross Aneurysms and Aortic Rupture in Mice Fed Sweet Peas or BAPN.

| Exp. | No. of mice | Mean initial wt, g | Mean wt at 28 da., g | Diet % | Deaths No. | Deaths Cause | Survivors killed At days | Survivors killed Autopsy | Total No. gross aneurysms |
|---|---|---|---|---|---|---|---|---|---|
| I | 9 | 13.7 | 25.1 | 50 sweet pea<br>10 casein | 2 | Ruptured aortas | 51 | 2 aneurysms<br>5 normal | 4 |
| | 9 | 14.1 | 24.2 | .2 BAPN • HCl | 1<br>1 | " "<br>Undetermined | 52 | 1 aneurysm<br>6 normal | 2 |
| | 6 | 13.6 | 24.9 | 50 edible pea<br>10 casein | 0 | | 52 | 6 " | 0 |
| | 7 | 12.4 | 23.1 | Rockland stock | 0 | | 52 | 7 " | 0 |
| II | 11 | 13.7 | 20.0 | .5 BAPN • HCl | 9 | Ruptured aortas | 76 | 2 " | 9 |
| | 11 | 13.6 | 17.6 | 1.0 " | 2<br>6 | " "<br>Undetermined* | 76 | 3 " | 5* |
| | 11 | 13.6 | 24.7 | Rockland stock | 0 | | 76 | 11 " | 0 |
| III | 15 | 12.5 | 22.7 | 50 sweet pea<br>10 casein | 1<br>1 | Ruptured aorta<br>Undetermined | 70 | 2 aneurysms<br>11 normal | 3 |
| | 11 | 12.7 | 22.2 | 50 sweet pea<br>20 casein | 3<br>1 | Ruptured aorta<br>Undetermined | 70 | 7 " | 3 |

"Normal" signifies absence of gross aortic aneurysms.

* Three of the 6 animals which died from undetermined causes in this group had gross aneurysms.

than that used by Lewis and Schulert gave no apparent protection.

Only mice receiving 0.5% and 1% levels of BAPN • HCl showed skeletal changes comparable to those occurring in rats receiving much lower levels of BAPN (Fig. 1). Spinal curvatures varied from slight to very severe. A few animals became severely crippled. Some animals survived 10 weeks of feeding, however, without showing symptoms and showed only minor skeletal changes at autopsy. Skeletal effects were therefore much more variable in mice than in rats, all of which rapidly develop severe skeletal changes at much lower levels of BAPN.

Deaths in groups receiving the 2 higher levels of BAPN • HCl (17 of 22 mice) occurred between the 20th and 75th day of the experiment. On the 1% level, mice which died within the first 6 weeks of the feeding period had severely deformed femurs. These animals also showed a greatly increased vascularity in periarticular areas and an abnormal red color of the long bones. Paraphymosis occurred in 5 of 11 mice at each of these levels of BAPN. Even though extreme paraphymosis and severe crippling occurred in some animals, no instance of paralysis or urinary retention was seen in any of the groups. The latter 2 symptoms are common in experimental lathyrism in rats.

The incidence of aortic aneurysms was also increased by higher levels of BAPN. The incidence of gross aneurysms is given in the Table. Deaths from aortic rupture occurred in all experimental groups. No ruptured aortas or gross aneurysms were found in any mice receiving either the Rockland or the edible pea control diets. Of the total of 26 gross aneurysms distributed throughout the experimental groups, 24 were located in the thoracic aorta. The remaining 2 were found in the upper lumbar area of the abdominal aorta.

*Aortic lesions. Microscopically,* the *normal mouse aorta* is similar to that of the rat. The intima is very thin, consisting mostly of endothelium. The media forms about 5/6ths or more of the entire wall and is well defined, consisting essentially of wavy elastic fibers, smooth muscle, and a small amount of collagen with fibrocytes. The adventitia is less well defined, relatively thin, and composed almost entirely of collagenous connective tissue with small numbers of rather delicate elastic fibers occurring in the inner portion.

Although not all aortas from experimental animals were examined, *histopathologically* no

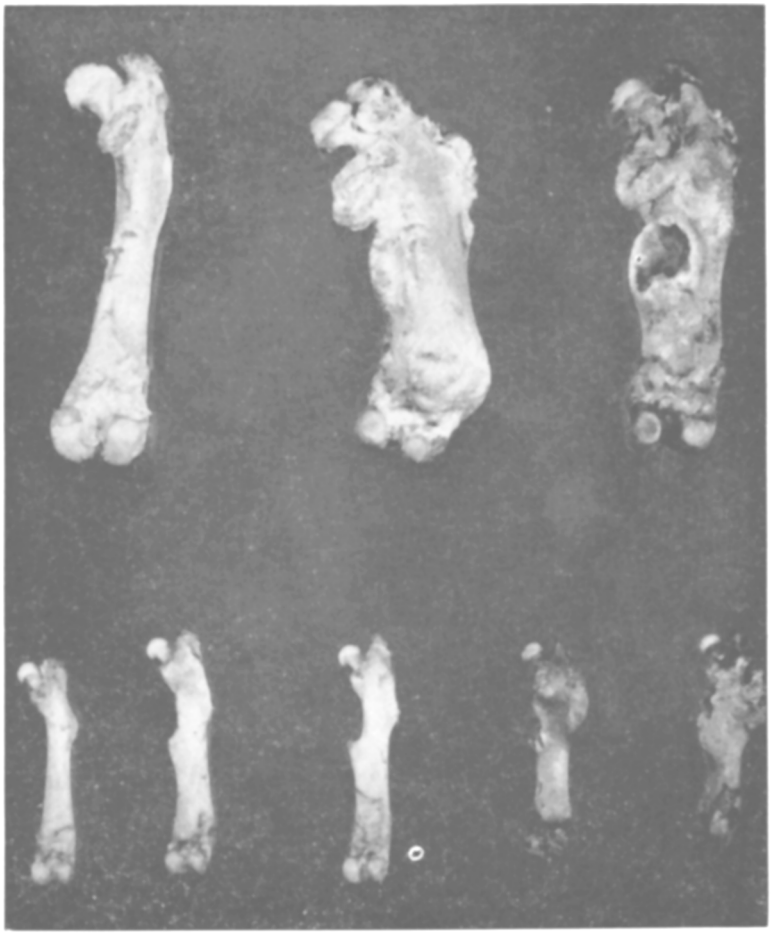

FIG. 1. Top row, rat femurs, left to right: normal; 50% sweet peas, 22 days; 0.25% BAPN • HCl, 15 days. Bottom row, mouse femurs, left to right: normal; 50% sweet peas, 50 days; 0.2% BAPN • HCl, 56 days; 0.5% BAPN • HCl, 28 days; 1% BAPN • HCl, 34 days. The rat femurs illustrate *typical* changes, the mouse femurs illustrate maximal changes in animals receiving above diets.

entirely normal aortas were found in any animals receiving sweet pea- or BAPN-containing diets.

No lesions of the *intima* were observed that appeared to be primary in nature. Proliferative changes were frequently observed but always appeared secondary to, or compensatory for, destructive lesions in the media. The early ones were quite cellular (endothelial and fibroblastic) and became more fibrous with increasing age of the lesion. In older aneurysms with thrombosis this intimal proliferation extended into the thrombus, aiding in its organization. Such organization was not extensive and seemed slow in its progress. Mural thrombi were observed only in cases with aneurysm formation. Recanalization of thrombi was not observed. Significant amounts of cholesterol or lipids were never observed.

Changes in the *media* seemed to be primary but their exact nature and sequence were often not readily apparent. The earliest change occurred between the elastic fibers and resulted in separation of these fibers. Initially the smooth muscle cells and fibrocytes between the elastic fibers seemed intact, indicating that the change was in the amount of ground substance. Whether this was simply an infusion of fluid, an edema, or a true increase in the amount of ground substance could not be determined by the methods em-

ployed. Following this, retrogressive changes were observed in the cells (myocytes and fibrocytes) between the elastic fibers. Varying numbers of these cells were destroyed resulting in a temporarily decreased cellularity of the media. The next change was in the elastic fibers and appeared to be a combination of swelling, fragmentation, and lysis which resulted eventually in their disappearance. When these medial changes were of sufficient extent, dilatation and aneurysm formation often resulted. Instances were observed, however, in which the medial damage appeared maximal and yet no aneurysm had resulted. Blood was frequently observed in the damaged media. Since no vasa vasorum were observed, it must be assumed that this blood entered the media through defects in the intima. Extension of blood into this damaged media often resulted in the classical picture of dissecting aneurysm. Severe retrogressive changes without bleeding often resulted in diffuse dilatation, fusiform aneurysm or saccular aneurysm formation. Any of these aneurysms may rupture with fatal hemothorax or hemoperitoneum. In some instances the retrogressive medial changes were repaired by formation of fibers having the staining characteristics of collagen. In no case were new elastic fibers or smooth muscle cells observed in these areas of medial repair. In one animal, fatal hemorrhage resulted from a small focal retrogressive lesion involving all layers of the aortic wall without significant dilatation or aneurysm.

Lesions of the *adventitia* seemed to be secondary rather than primary in that they were not observed in the absence of medial lesions. In cases of aortic dilatation or aneurysm formation without rupture, the adventitia often showed compensatory thickening. This was largely cellular or collagenous, depending on the age of the lesion, but in many cases there was an apparent increase of rather delicate elastic fibers. These elastic fibers did not seem to be derived from the media. Their increased prominence may have been due to the stretching and compression of loose periaortic tissues resulting from the dilatation. Blood was frequently observed infiltrating the adventitia. When the blood was moderate in amount, the adventitia and adjacent periaortic tissues proliferated to form a wall around it. In a few cases quite definite false aneurysmal sacs were formed in this manner. Blood in small amounts in either the media or adventitia was lysed or became organized.

*Summary.* 1) Weanling, male, albino mice were more resistant to the skeletal effects of diets containing sweet peas or β-aminopropionitrile (BAPN) than were rats. They also showed a much greater individual variation in susceptibility to the skeletal effects of such diets. At sufficiently high levels of BAPN, however, both skeletal and aortic lesions occurred. The microscopic changes occurring in the aorta are described in detail. 2) Paraphymosis also occurred, but the paralysis and urinary retention often seen in rats receiving similar diets were not observed.

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